BRAIN DAMAGE DUE TO ASPHYXIA: MECHANISM OF CAUSATION

Ronald E. Myers, M.D., Ph.D.

Why do the nerve cells of fetuses or newborns die or become destroyed as a consequence of their exposure to oxygen deficiency states? The oldest and still most widely accepted interpretation of the mechanisms that underlie both the loss of nervous system function and the development of brain injury with oxygen deprivation is a presumed deficiency in energy availability to the cells required to support vital cellular processes (2, 13). A similar mechanism has been posited to explain the death of cells in other parenchymal organs (3). However, recent studies from a number of laboratories have raised serious doubts that a deficit of energy availability can account for the loss of nervous system function with oxygen deficiency (1, 14) while other results obtained in our own laboratory have demonstrated a lack of correlation between the availability of high energy phosphate during exposure to a variety of oxygen deficiency states and the development of brain pathology. Rather, our results establish a close correlation between the tissue content of lactic acid at the end of exposure and whether or not brain injury will develop (8, 9, 10, 12). These results are now described.

A reduced oxygen availability to an animal or to man causes redox changes in the brain as a consequence of an impaired oxidation of cytochrome oxidase - the final link in the electron transport chain. An impaired oxidation of cytochrome oxidase, in turn, leads to an impaired oxidation of all the various antecedent links in the electron transport chain including nicotinamide adenine dinucleotide, the water soluble oxidation-reduction co-factor essential for many enzymatic transformations that take place in many metabolic pathways important for cellular bioenergetics. Thus, the altered redox state brought about by oxygen deficiency reduces the activity of the electron transport chain itself and increases both the absolute concentration and the proportion of available nicotinamide adenine dinucleotide that is present in its reduced state. An impaired electron transport is associated with major reductions in ATP production and, thus, major deficits in energy availability to the cell.

The maintenance of major portions of available nicotinamide adenine dinucleotide and the electron transport chain in a reduced state also leads to major impairments in citric acid cycle function because of a lack of hydrogen acceptors at the various dehydrogenase steps as one progresses from isocitrate, a-ketoglutarate, succinate, and malate. The maintenance of large amounts of nicotinamide adenine dinucleotide in the reduced state also inhibits the oxidative decarboxylation of pyruvate thereby interfering with the formation of acetyl-coenzyme A and further reducing citric acid cycle function. Rather, the presence of nicotinamide adenine dinucleotide primarily in the reduced state favors the reduction of pyruvic acid to lactic acid. As a consequence of all these alterations in biochemical function, all available carbohydrate in the tissue including that present as free

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glucose and that present as glucose polymerized to glycogen is converted to lactic acid. This process (of anaerobic glycolysis) is greatly accelerated by the loss of the breaking effect that is normally exerted by the cellular ATP when present at high concentrations acting at multiple points along the Embden-Meyerhoff pathway. The major consequence of this Pasteur effect is to increase the rate of carbohydrate breakdown to lactic acid by a factor as great as seven (4). The overall effect of all these processes is to lead to a near-total breakdown of all available free glucose and of glucose equivalents present in the form of glycogen to lactic acid over the first few minutes of exposure to circulatory arrest or to another form of anoxia.

The brain tissue of monkeys normally contains free glucose at a concentration of 3 to 5 umoles/g and polymerized glucose in the form of glycogen also at a concentration of 3 to 5 umoles/g. Taking into account the 1 to 3 umoles/g of lactic acid already present in the tissue, the conversion of all available tissue carbohydrate to lactic acid leads to a local accumulation of lactic acid to concentrations of 14 to 25 umoles/g (11, 15).

The complete oxidation of one molecule of glucose to 6 molecules of carbon dioxide and 6 molecules of water leads to a -686.0 kilocalories per mole free energy change. In contrast to this, the breakdown of the same glucose molecule to 2 molecules of lactic acid is associated with a free energy change of only -47.0 kilocalories per mole. Thus, converting available glucose to lactic acid rather than oxidizing it completely to carbon dioxide and water does lead to a marked energy deficit despite the considerable increase in rate of glycolysis brought about by the decreased availability of ATP and the disinhibition of specific enzymes at a variety of critical control points.

These considerations reveal that a reduced oxygen availability to tissue causes 1) a major reduction in energy availability to tissue to support vital cellular processes and 2) an accumulation of lactic acid both in the tissue and in the circulating blood. The first of these inferences is confirmed by finding marked reductions in the tissue contents of ATP and phosphocreatine as a result of exposure to any one of the oxygen deficiency states. Those investigators who have raised doubts whether such reduced energy availability can be incriminated as the cause for functional central nervous system disturbances developing during exposure to oxygen deficiencies point out these disturbances can develop under circumstances where the energy available to the brain in the form of high energy phosphate compounds has declined only to a minor degree (often after a reduction of less than 20%)(1, 14). However, those tissue circumstances that lead to pathologic change are readily and reproducibly separable and different from those that lead to disturbed function. Thus, it is quite possible to restore a normal nervous system function and to later demonstrate an intact brain in animals following exposures to periods to circulatory arrest or anoxia that far exceed those durations that are required to produce a complete but temporary loss of nervous system activity (5, 6, 7).

Studies we have carried out over the last several years in collaboration with Dr. Michio Yamaguchi have pointed out a lack of correlation between the behavior of the high energy phosphate compounds as exemplified by the levels of ATP present in the brain tissue and the subsequent development of brain pathology whether or not the brain subsequently develops pathology or not. In a first study, rhesus monkeys were exposed to 10 minute episodes of circulatory arrest (11). Some of the animals were previously infused with physiological saline solutions while others were infused with 25% glucose solutions leading to elevations of their serum glucose concentrations to values as high as 650 mg %. Prior studies have clearly indicated that monkeys that have been food-deprived for 24 hours and infused with saline solutions can undergo exposure to periods of circulatory arrest that last for as long as 14 minutes without developing any neurologic abnormalities or any gross or microscopic findings of brain damage while similar animals infused with glucose solutions leading to only slight elevations of their serum glucose concentrations undergo a grave neurological deterioration beginning several hours after they are resuscitated and terminating in their deaths in 100% of cases many hours later (12). The bar graphs depicted in Figure 1

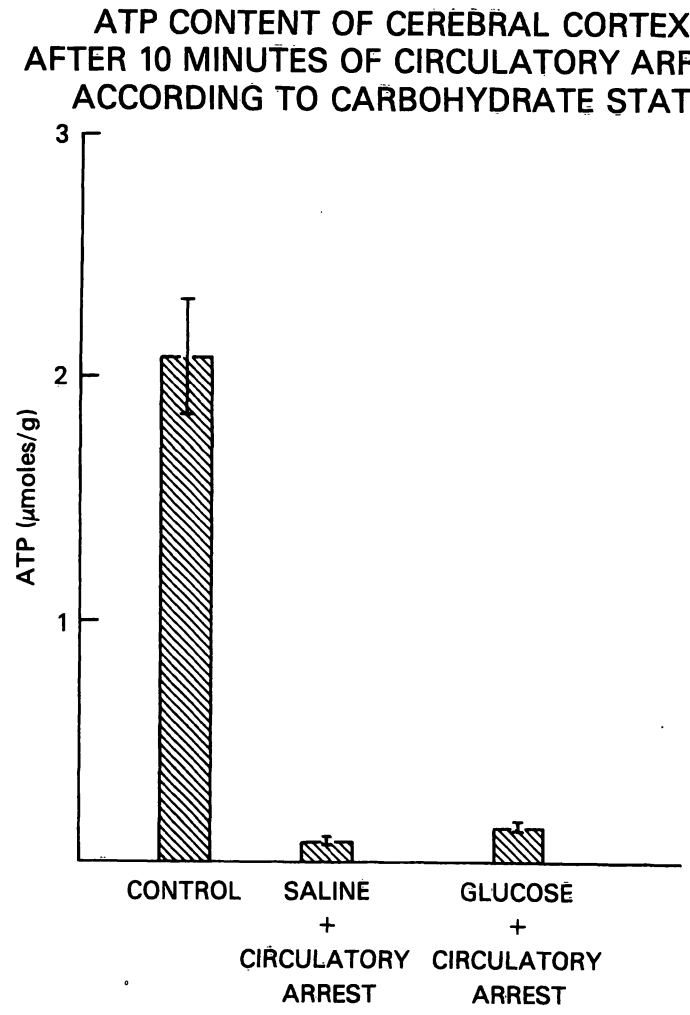

Fig. 1: Brain tissue concentrations of adenosine triphosphate (ATP) in food-deprived control animals and in animals exposed to 10 minutes of circulatory arrest following pretreatment with saline or glucose infusions (Myers and Yamaguchi, 1976).

indicate the food-deprived monkeys that were infused either with saline or with glucose solutions before they were exposed to circulatory arrest both experienced major reductions in their cerebral cortical ATP contents as measured at the termination of exposure. The reductions in ATP were significantly greater in the animals pretreated with saline than in those pretreated with glucose infusions even though the former would be expected to survive brain-intact while the latter, in 100% of cases, would be expected to undergo a grave neurologic deterioration and die with major widespread brain pathology. These findings show a lack of correlation between the magnitude of reduction in the ATP content of the cerebral cortex and whether or not the animals subsequently develop brain pathology.

A similar lack of correlation between the extent of reductions in high energy phosphate during exposure to oxygen deficiency and appearance of brain pathology is illustrated in the bar graphs of Figure 2. Food-deprived rhesus monkeys were

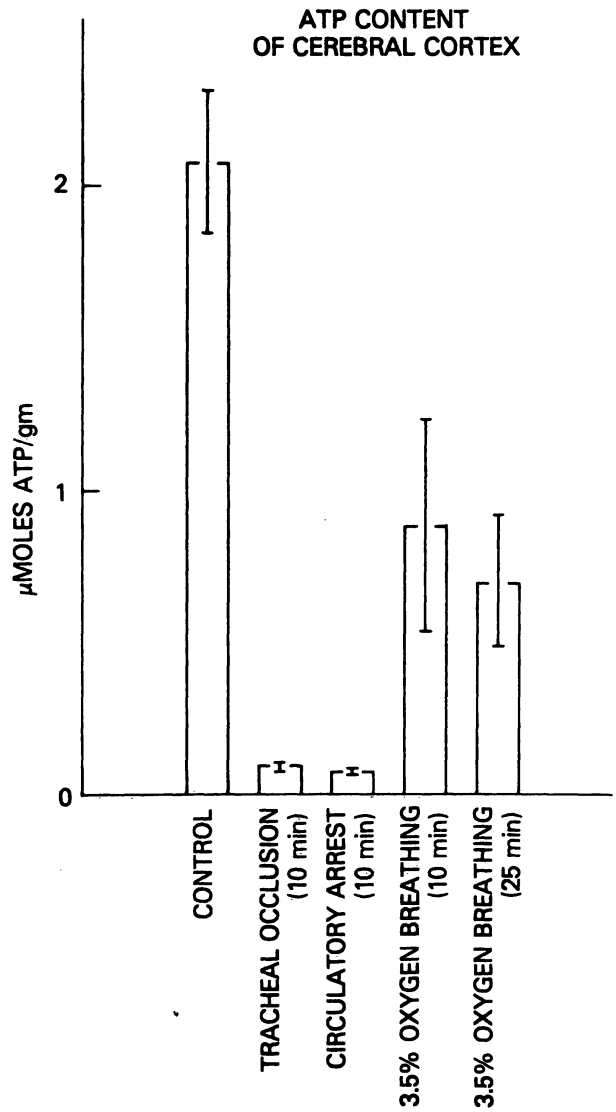

Fig. 2: Adenosine triphosphate (ATP) contents of cerebral cortex of young adult control monkeys and of monkeys after exposure to 10 minutes of tracheal occlusion (anoxia), 10 minutes of circulatory arrest (anoxia), and 10 and 25 minutes of 3.5% oxygen breathing (marked hypoxia) (Yamaguchi and Myers, 1976).

exposed to 4 different conditions of oxygen deprivation including: 1) 10 minutes of tracheal occlusion, 2) 10 minutes of circulatory arrest, 3) 10 minutes of breathing 3.5% oxygen in nitrogen and 4) 25 minutes of breathing the same hypoxic gas mixture (11). Prior work in our laboratory has demonstrated that only the animals of category 4 exposed to 25 minutes of marked hypoxia will develop neurologic abnormalities and many will die several hours later with brain edema or survive but will show focal brain injury. Exposure to 10 minutes of tracheal occlusion or of circulatory arrest both of which are well tolerated by the food-deprived animals depresses the cortical tissue ATP contents far more than does exposure to either 10 or 25 minutes of marked hypoxia. Thus, once again, the development of brain pathology fails to follow the behavior of high energy phosphate compounds. Furthermore, the animals exposed to marked hypoxia for 10 and 25 minutes, though they show no significant differences in their cortical ATP contents during exposure and at the termination of exposure, they do show marked differences in brain pathologic outcome. The animals exposed for 10 minutes of hypoxia survive and do well while those exposed for 25 minutes develop marked brain injury and die in large proportion. Thus, in the number of circumstances depicted in Figures 1 and 2 no correlation could be demonstrated between the ATP content of the cerebral cortex during exposure to oxygen deficiency and whether or not the animals would develop cortical injury.

In contrast to this lack of correlation between the behavior of tissue ATP and the development of brain pathology is the circumstance of lactic acid and its accumulation in the brain during exposure to the oxygen deficiency states. The extents to which lactic acid accumulates in the brain tissue in the animals earlier described which were food-deprived for 24 hours, given infusions of saline or glucose solutions, and then exposed to 10 minutes of circulatory arrest is described in the bar graphs of Figure 3 (11). The animals pretreated with saline infusions and exposed to 10 minutes of circulatory arrest (all of which would survive brain-intact) accumulated lactic acid to an average concentration of 12 umoles/g. tissue. In contrast, the animals pretreated with glucose infusions (all of which would later undergo a grave neurologic deterioration and die with brain edema and widespread necrosis) accumulated lactic acid to concentrations in excess of 30 umoles/g. The basis for this marked difference in behavior with respect to lactic acid accumulation relates to the carbohydrate state of the animals and their prior history of food intake or of infusion of glucose. The infusion of glucose solutions or the recent ingestion of a meal high in carbohydrates significantly increases the brain content of free glucose. The exposure to circulatory arrest or to total asphyxia causes all available free glucose and also all glycogen in the brain tissue to break down to lactic acid. The extent to which lactic acid accumulates in the brain under these circumstances is stoichiometrically related to the availability of carbohydrate in all these forms in the brain. For this reason, the prior feeding of carbohydrate or the infusion of glucose solutions into the animals proportionally increases the extent to which lactic acid accumulates in the tissue.

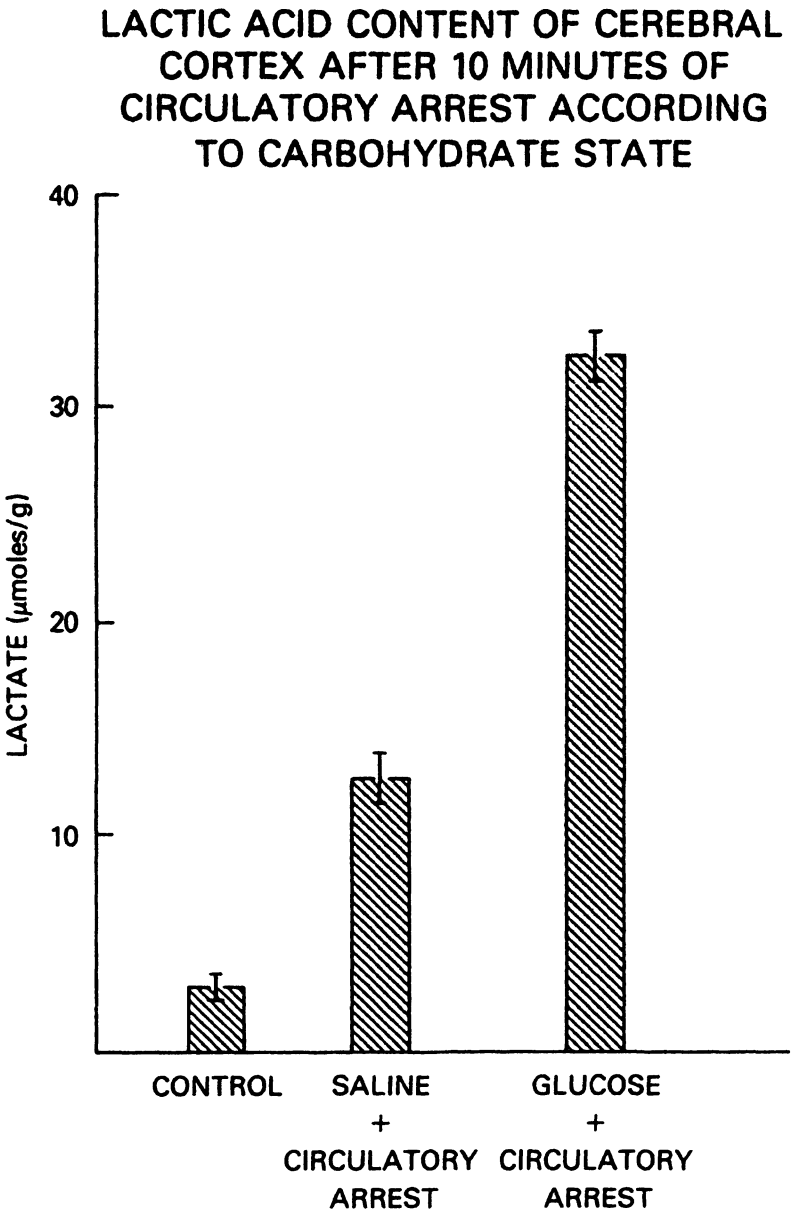

Fig. 3: Brain tissue concentrations of lactate in food-deprived control animals and in animals exposed to 10 minutes of circulatory arrest following pretreatment with saline or glucose infusions (Myers and Yamaguchi, 1976).

The behavior of lactic acid in the brain tissue in the animals exposed to the 2 types of anoxia or the 2 durations of marked hypoxia as described above is illustrated in the bar graphs of Figure 4 (16). The exposure of food-deprived monkeys to 10 minutes of circulatory arrest or of tracheal occlusion both of which is well tolerated causes lactic acid to accumulate in the cerebral cortex only to 10 to 12 umoles/g. Likewise, the exposure of animals to 10 minutes of marked hypoxia (3.5% oxygen breathing) also causes lactic acid to accumulate in the cortex to mean values close to 10 umoles/g and such an exposure is also well tolerated by these animals. However, the exposure of animals to 25 minutes of

marked hypoxia which will cause many to die with brain edema, causes the lactic acid content of the brain tissue to augment to a mean value of 24 umoles/g. Thus, in 2 quite separate circumstances, e.g., exposure of glucose-infused animals to anoxia and exposure of any animals to a prolonged hypoxia, the accumulation of lactic acid in brain tissue at high concentrations is associated with the development of a marked brain pathology.

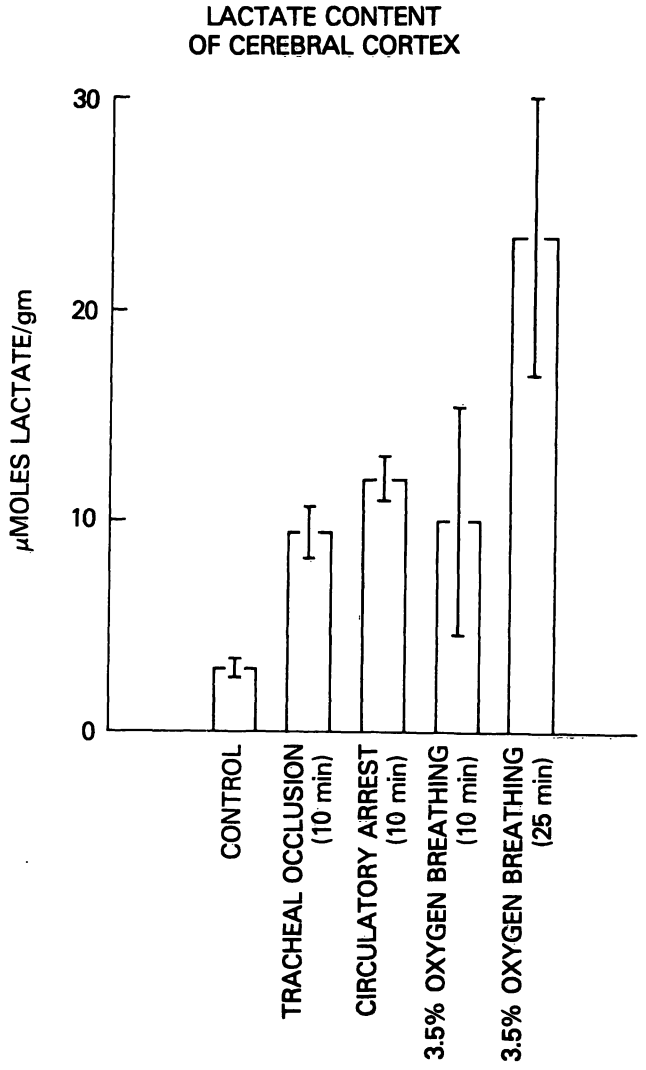

Fig. 4: Lactate contents of cerebral cortex of young adult control monkeys and of monkeys after exposure to 10 minutes of tracheal occlusion (anoxia), 10 minutes of circulatory arrest (anoxia), and 10 and 25 minutes of 3.5% oxygen breathing (marked hypoxia)(Yamaguchi and Myers, 1976).

Conclusions: The results of our present studies fail to show any correlation between the magnitude of the energy deficit of the brain tissue as defined by its ATP contents at the termination of exposure to some form or other of oxygen deprivation and the later behavior of the brain with respect to appearance of edema or development of pathology. Rather, our studies point out a close correlation between the accumulation of lactic acid in the brain at high concentrations (>18 to 20 umoles/g) and the occurrence of brain edema, widespread tissue necrosis, and death of the animals. It is inferred that the critical determiner of brain pathologic outcome in relation to exposure to the various oxygen deficiency states is the extent to which lactic acid accumulates in the brain tissue. The extent to which lactic acid accumulates in these circumstances, in turn, is determined by the animals' carbohydrate state or their history of food intake at the time of exposure.

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MYERS, Ronald E., M.D., Ph.D.
Chief
Laboratory of Perinatal Physiology
National Institutes of Health
Park Building, Room 451G
Bethesda, Maryland 20205 / USA